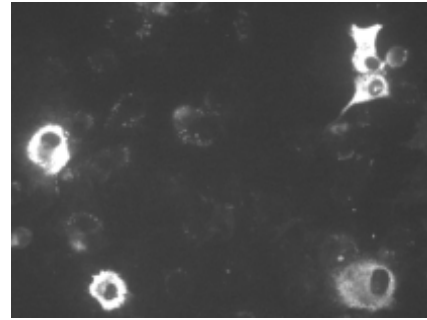
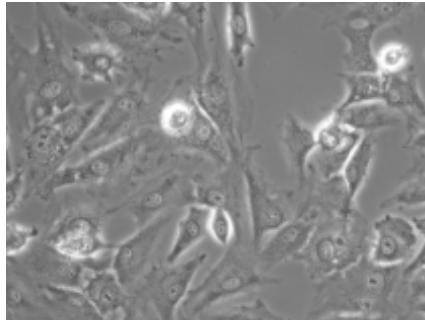


Targeting Systems Introduces :
Targefect-Chondrocyte



Porcine Chondrocytes were transfected with a construct encoding a domain of aggrecan core protein tagged with GFP. When expressed in porcine chondrocytes and other cell types, the protein localizes in the ER.

Transfection was performed as follows :

In 125 µl of HG-DMEM (no serum), 0.8 µg of DNA was added, followed by 3 µl of Targefect and 6 µl of Virofect. The complex was incubated at 37°C for 25 minutes, and mixed with 1 ml culture medium (HG-DMEM/F12 1:1 with 10% serum), then added to the cells on cover slips in a 35 mm dish. In order to remove proteoglycans that interfere with transfection, prior to transfection chondrocytes were treated with 250 I.U. purified testicular hyaluronidase in 1.5 ml OptiMeM at 37°C for 30 minutes. The transfection complex was removed 2 hours later and replaced with fresh culture medium. Cells were incubated overnight.

A transfection rate of 20-30% was achieved using this protocol. Data courtesy of Dr. and Dr. Barbara Vertel, Department of Cell Biology and Anatomy, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Rd. North Chicago, IL 60064

Suggested Transfection Protocol

The Targefect-Chondrocyte Kit is a combination of the Targefect-Chondrocyte reagent with the Virofect enhancer for optimal transfection of primary Chondrocytes. Set up cells to be transfected so that they are about 70% confluent at the time of the experiment.

Prepare transfection complexes as follows :

- Use clear plastic tubes for complex formation.
- Complexing conditions for formation of transfection complexes:
- Use high glucose DMEM (Dulbecco's modified eagle's medium containing 4500 mg/liter glucose).

Tube #	High Glucose DMEM	DNA	Targefect Chondrocyte	Virofect
1	0.5 ml	6 µg	12 µl	-
2	0.5 ml	6 µg	12 µl	25 µl
3	0.5 ml	4 µg	12 µl	25 µl

Add DMEM first, then add DNA, mix well by flicking the tube about 12 times to create a vortexing action. Add Targefect next, mix well again by flicking the tube. Add Virofect last and mix again. Incubate the tubes at 37° C for 25 minutes to form the transfection complexes.

For a 12-well dish add 125 µl of transfection complex to 1 ml of complete media per well.

Add 0.25 ml of the transfection mix to 2 ml of complete media for 1 well for a 6-well dish (or for a 35 mm dish). Prepare 0.5 ml of transfection complex per 60 mm dish and 1.25 ml of transfection complex per 100mm dish. Swirl the dish to mix transfection complexes with the cell culture media (with 5-10% serum). Incubate overnight at 37° C in CO2 incubator. Aspirate media containing transfection complexes 3 hrs after transfection with complete chondrocyte growth media.

Virofect enhancer : Please store at -70° C on arrival.

Targefect Chondrocyte : Please mix the reagent gently by inverting the tube (do not vortex) and then store at 4° C upon arrival.

The expected efficiency is 20-40%. This kit (catalog #CHON-01) contains sufficient reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes.

Targefect-SMC Catalog #	Quantity	T.S. list Price
CHON-01	1	\$250
CHON-10	10	\$2000

Contents : Two solutions
Targefect-Chondrocyte and Virofect (600 µl each)